Original Article

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Pseudobasophilia in Pediatric Age Group

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Abstract

Background: Automated instruments show elevated basophil counts in some cases in which the peripheral smear examination does not reveal any basophilia. This study tries to analyze the correlation between the pseudobasophilia in children of age ≤9 years, reported by the hematology auto analyzers, AcT.5Diff and HmX, and the coexisting hematological abnormalities, if any.

Samples and Methods: A sample of 70 cases and an equal number of controls matched for age group, machine and sample processing day were included in this study. The peripheral smear of each case was examined and 500 cell differential leukocyte counts were made. In 16 of the 70 cases, absolute basophil count was calculated manually. Analysis of the parameters such as age, sex, time taken to process the sample, percentage and absolute count of basophils automated as well as manual, any abnormality flagged by the auto analyzers and abnormalities detected on peripheral smear examination was done. The mean, standard deviation, standard error, 95% confidence interval were computed for cases and controls. To test the equality of two means, Independent samples *t*-test was used and to compare two groups based on categorical data, Mann–Whitney tests were applied. Statistical analysis was performed using IBM SPSS Version 21 software.

Results: The basophilia reported by the automated instruments in the cases was found to be spurious. The $P < 0.05$ with pseudobasophilia in cases of neonates as compared to the other age groups; in cases having Hb ≥ 15 g/dl, nucleated red blood counts (nRBC), neutrophilia, neutrophilic shift to left, reactive lymphocytes, lymphocytosis, standing time ≥ 2 h.

Conclusions: The associations of pseudobasophilia with neonatal age, Hb ≥ 15 g/dl, nRBCs, neutrophilia, neutrophilic shift to left, reactive lymphocytes, lymphocytosis and prolonged standing time are significant.

Key words: Basophils, Child, Hematology, Leukocyte disorders

INTRODUCTION

Automated instruments show elevated basophil counts in some cases in which the peripheral smear examination does not reveal any basophilia. This discrepancy is evaluated and corrected in many laboratories before the final report is issued. However, in certain instances, physicians do get false results though these may not have an impact on the health care given to the patients.[1-3] Studies have proven that basophils are not increased in the count during childhood and the counts are comparable to those of adults.[4,5] Further, hematology automated counters are expected to give relatively precise results, as they count some thousand leucocytes; however, studies have shown a low correlation between the basophil count of various instruments, as well as between instruments and the reference method.[2,6] We have observed auto analyzers giving falsely elevated basophil percentage i.e. pseudobasophilia especially in the pediatric age group. In this study, the basophil count given by the hematology auto analyzer is counterchecked by peripheral smear examination and manual basophil counts. Though, many stains including toluidine blue, neutral red, alcian blue can be used to stain the basophils, toluidine blue is found to be particularly useful and hence it is used in this study to do manual basophil count.[7,8] This study tries to analyze the correlation between the pseudobasophilia in children of age less than or equal to 9 years, reported by the hematology automated instruments, AcT.5Diff and HmX, and the coexisting hematological abnormalities, if any.

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Objectives

This study attempts to cross check automated counter (Beckman Coulter Act.5 diff and Beckman Coulter HMX) generated basophilia by peripheral smear examination and to assess the hematological factors significantly associated with pseudobasophilia.

SAMPLES AND METHODS

Cases of age ≤9 years, which fulfill both the following criteria: (i) Automated counter generated basophil percentage of ≥2% of white blood counts (WBCs) (on two successive readings), (ii) peripheral smear basophil differential count <2% (when the WBC differential count is done on 500 cells by two independent observers) and controls matched for age group, automated instrument in which the sample was processed and the day of processing of sample, which show basophil percentage of <2% in both the automated results and the peripheral smear examination were included in the study.

Cases and matched controls whose automated results and the peripheral smear examination are concordant with the diagnosis of basophilia were excluded.

A sample of 70 cases and an equal number of controls, as defined by the inclusion and exclusion criteria during a one year period from December 2012 to November 2013 were included in the study. The study had been approved by the Institutional Ethics Committee. As the study was done only on the samples submitted for routine laboratory investigations, consent was not obtained from the patients' and the controls' attenders. The auto analyzers (AcT.5 Diff and HmX) were being calibrated periodically as recommended; commercial quality control samples were being run once every day and integration of the values of the patient samples was being checked every day. The coefficient of variation (CV%) calculated for absolute basophil counts using commercial quality control samples run on 20 consecutive days once daily in each counter was 0.00% and 2.46% for HmX and AcT.5Diff respectively.

The peripheral smear of each case was examined by two independent observers (Dr. Femela Muniraj and Dr. Vijay Amritraj) and 500 cell differential leucocyte counts were made.

In 16 out of the 70 cases, absolute basophil count was calculated manually, using the following staining and diluting fluid, which is a modification of that used by Moore and James.[5,6]

0.05% toluidine blue in 0.9% saline - 10 ml
95% ethyl alcohol - 2.75 ml
Saturated solution of saponin in 50% ethyl alcohol - 0.25 ml.

The blood sample of the cases anti-coagulated with ethylenediaminetetraacetic acid was diluted with the fluid mentioned above, in the ratio of 1:10, since basophils are relatively fewer in number. Improved neubauer chamber was charged with this sample, ensuring that both the chambers in the hemocytometer were charged. The hemocytometer was allowed to stand for a few minutes to allow the leukocyte to settle. With this fluid, the basophils were stained reddish-violet in color, whereas the nuclei of other leukocyte stained bluish-violet. Since, the dilution factor, the fluid used, and the chambers used, were the same for both the basophil count and the total leukocyte count (TLC), both were calculated simultaneously.

Eight corner squares (having 16 smaller squares each) (each measuring 1 mm × 1 mm × 0.1 mm) from two chambers (each having four corner squares) were used for counting both the basophils and the TLC.

Dilution factor = 10
Number of squares counted = 8
Dimension of each square = 1 mm × 1 mm × 0.1 mm
Depth of the chamber = 0.1 mm
n = Number of basophils/total leukocytes in eight corner squares

Absolute basophil count/total leukocyte
count = (n × 10)/(8 × 0.1) = n × 12.5 per cubic mm

Analysis of the parameters such as age, sex, time taken to process the sample, percentage and absolute count of basophils automated as well as manual, any abnormality flagged by the auto analyzer and abnormalities detected on peripheral smear examination was done. Summary statistics such as the mean, standard deviation, standard error, 95% confidence interval were computed for cases and controls. To test the equality of two means, independent samples *t*-test was used and to compare two groups based on categorical data, Mann-Whitney tests were applied. Statistical analysis was performed using International Business Machines Statistical Package for the Social Sciences Version 21 software.

RESULTS

The mean percentages of basophils on automated counters (AcT.5Diff and HmX) for cases and controls are 7.973 (95% confidence interval [CI] = 6.511-9.435) and 0.851 (95% CI = 0.728-0.974) respectively; the mean difference is 7.122 and the $P < 0.001$. The mean absolute count of basophils on automated counters (AcT.5Diff and HmX) for cases and controls are 1160.143 per cubic mm (95% CI = 907.217-1413.069 per cubic mm) and 109.571

per cubic mm (95% CI = 88.405-130.737 per cubic mm) respectively; the mean difference is 1050.572 and the $P < 0.001$. The mean percentage of basophils on peripheral smear of the cases is 0.191% (95% CI = 0.122-0.260). The mean of the absolute counts of basophils counted manually in the 16 cases is 27.344 per cubic mm (95% CI = 13.159-41.529 per cubic mm). The $P = 0.002$ and the range is between 0 and 87.5 per cubic mm. The difference between the means of absolute counts of basophils calculated by the automated counter and the manual method for those 16 cases is 1038.29 (Table 1).

Correlation with Machine Model

AcT.5Diff hematology auto analyzer reported basophilia in the majority (90%) (63/70) of the cases, and the rest (10%) (7/70) was reported by the HmX hematology auto analyzer; $P < 0.001$ (Table 2).

Correlation with Age

The majority (67.1%) of the cases (47/70) belong to the neonatal age group (0-28 days of age), out of which 40/70 cases were 0-1 day old babies; whereas, among the controls, 15.7% (11/70) were neonates. The mean age of the cases is 14.11 days and that for the controls is 36.50 days; $P = 0.049$ (Table 2).

Correlation with Sex

42/70 (60%) of the cases were males, 28/70 (40%) were females. 39/70 (55.7%) and 31/70 (44.3%) of the controls were males and females respectively; $P = 0.608$ (Table 2).

Correlation with Red Blood Cells (RBCs)

RBCs were macrocytic normochromic in 45/70 (64.3%) of the cases and 9/70 (12.8%) of the controls; microcytic hypochromic in 5/70 (7.1%) of the cases and 13/70 (18.6%) controls; normocytic normochromic in 20/70 (28.6%) cases and 48/70 (68.6%) controls; the $P < 0.001$. Hemoglobin was ≥ 17g/dl in 28/70 (40%) of cases and 7/70 (10%) of controls; $P < 0.001$. Of these, 24 cases and 6 controls were 0 to 1 day old. Hemoglobin was ≥15 g/dl, but <17 g/dl in 14.3% (10/70) cases and 1.4% (1/70) controls; $P = 0.005$; out of these, 7/10 cases and 0/1 control were 0-1 day old. Nucleated red blood count (nRBCs) were present in 27/70 (38.6%) of cases and were not seen in case of controls; $P < 0.001$ (Table 2).

Correlation with Leukocyte

Reactive lymphocytes were present in the peripheral smears of 23/70 (32.9%) cases and in 3/70 (4.3%) controls; the $P < 0.001$. Neutrophilia (relative and absolute) was seen in 4/70 (5.7%) cases and 18/70 (25.7%) controls; $P = 0.001$. Neutrophilic shift to left was present in 19/70 (27.1%) cases and 2/70 (2.9%) controls; $P < 0.001$. Lymphocytosis was seen in 15/70 (21.4%) cases and 5/70 (7.1%) controls; $P = 0.025$. Leukocytosis was seen in 10/70 (14.3%) cases and 14/70 (20%) controls; leukopenia in 3/70 (4.3%) cases and 2/70 (2.9%) controls; the count was normal in 57/70 (81.4%) cases and 54/70 (77.1%) controls; $P = 0.578$. Eosinophilia was observed in 5.7% (4/70) cases and 5.7% (4/70) controls; $P = 1.000$ (Table 2).

Correlation with Platelet Count

54/70 (77.1%) cases and 60/70 (85.7%) controls had normal platelet count. Thrombocytopenia was observed in 13/70 (18.6%) of cases, and 3/70 (4.3%) of controls; thrombocytosis in 3/70 (4.3%) of cases, and 7/70 (10%) of controls; the $P = 0.122$ (Table 2).

Table 1: Descriptive analysis of Basophils

Parameter	% of basophils as per counter (*n*=70)	Absolute count of basophils as per counter (per cu.mm) (*n*=70)	% of basophils on PS (*n*=70)	Manual absolute count of basophils (*n*=16)
Cases				
Mean	7.973	1160.143	0.191	27.344
Median	4.750	690.000	0.000	12.500
95% CI				
Lower	6.511	907.217	0.122	13.159
Upper	9.435	1413.069	0.260	41.529
SD	6.240	1079.656	0.292	28.946
Min	2.1	50	0	0
Max	27.9	4590	1.2	87.5
Controls				
Mean	0.851	109.571	-	-
Median	0.800	80.000		
95% CI				
Lower	0.728	88.405		
Upper	0.974	130.737		
SD	0.530	90.353		
Min	0	0		
Max	1.9	350		

Min: Minimum, Max: Maximum, SD: Standard deviation, CI: Confidence interval

Table 2: Descriptive analysis of other parameters

Parameter	Cases		Controls		*P* value
	Frequency	Percent	Frequency	Percent	
Descriptive analysis of age (*n*=70)					
NB (0-28 days)	47	67.1	11	15.7	0.049
29 days-9 years	23	32.9	59	84.3	
Descriptive analysis of gender (*n*=70)					
Female	28	40	31	44.3	0.608
Male	42	60	39	55.7	
Descriptive analysis of machine model (*n*=70)					
HmX	7	10	7	10	0.000
AcT 5Diff	63	90.0	63	90.0	
Descriptive analysis of RBC predominant morphology (*n*=70)					
Microcytic hypochromic	5	7.1	13	18.6	0.000
Macrocytic normochromic	45	64.3	9	12.8	
Normocytic normochromic	20	28.6	48	68.6	
Descriptive analysis of Hb≥17 g/dl (*n*=70)					
Yes	28	40	7	10	0.000
No	42	60	63	90	
Descriptive analysis of Hb≥15 g/dl, <17 g/dl (*n*=70)					
Yes	10	14.3	1	1.4	0.005
No	60	85.7	69	98.6	
Descriptive analysis of nRBCs (*n*=70)					
Present	27	38.6	0	0	0.000
Absent	43	61.4	70	100	
Descriptive analysis of total leucocyte count (*n*=70)					
Normal count	57	81.4	54	77.1	0.578
Leukocytosis	10	14.3	14	20	
Leukopenia	3	4.3	2	2.9	
Descriptive analysis of neutrophilic shift to left (*n*=70)					
Present	19	27.1	2	2.9	0.000
Absent	51	72.9	68	97.1	
Descriptive analysis of neutrophilia (*n*=70)					
Present	4	5.7	18	25.7	0.001
Absent	66	94.3	52	74.3	
Descriptive analysis of eosinophilia (*n*=70)					
Present	4	5.7	4	5.7	1.000
Absent	66	94.3	66	94.3	
Descriptive analysis of lymphocytosis (relative/absolute) (*n*=70)					
Present	15	21.4	5	7.1	0.025
Absent	55	78.6	65	92.9	
Descriptive analysis of reactive lymphocytes on PS (*n*=70)					
RL	23	32.9	3	4.3	0.000
Nil	47	67.1	67	95.7	
Descriptive analysis of platelets on PS (*n*=70)					
Thrombocytopenia	13	18.6	3	4.3	0.122
Thrombocytosis	3	4.3	7	10	
Adequate	54	77.1	60	85.7	
Descriptive analysis of standing time of sample ≥2 h (*n*=70)					
Yes	15	21.4	0	0	0.000
No	55	78.6	70	100	

RBC: Red blood count

Correlation with Standing Time

The mean standing time of the samples of the cases and controls is 88.014 min (95% CI = 68.334-107.694 min) and 21.871 min (95% CI = 19.515-24.227 min) respectively. The standing time of the sample before being fed into the auto analyzer was more than 1 h and 2 h in 38/70 (54.3%) and 15/70 (21.4%) of the cases respectively; none of the control samples were standing for more than 1 h; the maximum time being 45 min; $P < 0.001$ (Table 2).

DISCUSSION

Beckman Coulter uses Volume, Conductivity, and Scatter technology for differential counting of WBCs. AcT.5diff analyzer uses sequential dilution system, in which the basophils as well as the other WBCs are analyzed simultaneously in the "WBC/Basophil bath" (WBC/Baso bath). The basophils are differentiated from other leucocytes using specific cell lysis, impedance

technology and histogram thresholds. Focused flow impedance is used for the measurement of the volume and absorbance cytochemistry to analyze the internal stained structure of the leucocytes. Counting of basophils employing light scatter is considered to be more specific in terms of lesser frequency of pseudobasophilia.[7,8]

According to Clinical and Laboratory Standards Institute, for cells fewer than 5% of the total leucocyte count, manual count cannot be considered as a reference method. Automated hematology instruments are considered to give results with better precision, compared to manual methods, owing to the great difference in the number of cells counted, but as yet, have not been proved to be reliable.[7]

Moore and James tested the various metachromatic stains for counting basophils and found toluidine blue to be the most suitable. He used the following staining and diluting fluid for absolute basophil count:[5,6]

0.05% toluidine blue in 0.85% saline - 40 ml
95% ethyl alcohol - 11 ml
Saturated solution of saponin in 50% ethyl alcohol - 1 ml

Mitchell modified this fluid and used for counting the basophils in the capillary blood of new-born infants. He used 0.075% toluidine blue instead of 0.05% and he further reduced the volume of 95% ethyl alcohol to half i.e., 5.5 ml.[4,5] In this study, the basophils could be identified easily and differentiated from the other leucocytes with the use of this fluid.

In the study by Mitchell on 20 healthy term new-born infants, circulating basophil count increased after birth reaching a maximum at 24 h (mean absolute basophil count = 52 per cubic mm; range = 13-35 per cubic mm) in 17 of the 20 infants. The count fell down between 1 and 3 days reaching a minimum on 5th day; the count increased again reaching a value at 6 weeks that is twice that recorded at 1 week.[4] In his another study on 67 healthy children in the age range of 6 months to 12 years, the mean absolute count of basophils was 45 $\pm$ 2.5 per cubic mm.[5] In the present study, pseudobasophilia was found significantly in the neonatal period, especially during 0-1 day of birth. This is in concordance with the observation made by Mitchell, who confirmed that basophils are numerous in the new-born period, reaching a peak in 24 h, and declining thereafter.[4]

Gender difference does not have any statistical significance among the cases as well as the controls. Even in adults, absolute basophil counts do not differ between males and females, as observed by Moore and James.[6]

AcT.5Diff auto analyzer reported basophilia in more number of cases in this study as compared to HmX counter and the difference is statistically significant. This is because, AcT.5Diff employs absorbance cytochemistry and volume to give a differential leucocyte count and the cytoplasmic granules of only the neutrophils, eosinophils, monocytes are stained by the fix reagent leaving out the basophils. The HmX auto analyzer uses light scatter along with volume and conductivity to analyze the surface characteristics and internal structure of the cell.[7,8]

In our study, we found a statistically significant association of pseudobasophilia with cases which had a hemoglobin level of $\geq$ 15 g/dl; more so in cases with Hb $\geq$ 17 g/dl. WBC differential counting in auto analyzers is done after RBC lysis. Automated counters are known to give erroneous WBC counts and differentials in case of inadequate lysis of the RBCs.[9] The presence of nRBCs only in the cases and not in the controls and the high value of hemoglobin seen in more number of cases compared to the controls are related to the age. A report of pseudobasophilia by the auto analyzer can be explained by the fact that WBC counts including differentials are done after lysis of the RBCs. RBC morphology did not differ significantly between the cases and the controls. Most of the cases had macrocytic RBCs and most of the controls had normocytic normochromic RBCs because of the age factor, that is, most of the cases were neonates.

Neutrophilic shift to left and neutrophiliawere significantly associated with the pseudobasophilia. Eosinophilia did not have statistically significant association with pseudobasophilia. Eosinophilia was not found to be associated even with the true basophilia in the study by Mitchell.[5] The granules, whether exhibiting toxic change or not, are misinterpreted as that of basophils. Pseudobasophilia was observed in cases which showed reactive lymphocytes in the peripheral smear. Automated counters are known to misinterpret pathologic leucocytes as basophils. Sysmex (Sysmex, Kobe, Japan), ADVIA (Siemens Healthcare Diagnostics, Deerfield, IL), and Horiba (Horiba Medical, Kyoto, Japan) instruments report spurious basophilia, when pathologic leucocytes are present in the sample.[7] Pseudobasophilia in the presence of reactive lymphocytes has already been documented and the finding in this study is in accordance with it.[2,10] Lymphocytosis being significantly associated with pseudobasophilia can be explained by the fact that reactive lymphocytes are seen in many cases of lymphocytosis.

Platelet count did not have any significant association with pseudobasophilia in the present study.

Prolonged standing time had been significantly associated with pseudobasophilia and it is a documented cause of spurious basophilia.[11,12]

CONCLUSION

In this study, spuriously elevated basophil counts were given by the automated counters AcT.5Diff and HmX in the cases and their associations with neonatal age group, Hb ≥ 15 g/dl, nRBCs, neutrophilia, neutrophilic shift to left, reactive lymphocytes, lymphocytosis and prolonged standing time are significant.

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